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Original Research

Pyridoxal 5′-phosphate alleviates prenatal pyridaben exposure-induced anxiety-like behaviors in offspring

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ABSTRACT

Pyridaben (PY) is a widely used organochlorine acaricide, which can be detected in the peripheral blood of pregnant women. Available evidence suggests that PY has reproductive toxicity. However, it remains uncertain whether prenatal PY exposure impacts neurobehavioral development in offspring. Here, we administered PY to pregnant mice at a dose of 0.5 and 5 mg kg^{-1} day^{-1} via gavage and observed anxiety-like behaviors in PY offspring aged five weeks. We then integrated the metabolome and transcriptome of the offspring's brain to explore the underlying mechanism. Metabolome data indicated that the vitamin B6 metabolism pathway was significantly affected, and the pyridoxal 5′-phosphate (PLP) concentration and the active form of vitamin B6 was significantly reduced. Moreover, the transcriptome data showed that both PLP generation-related *Pdxk* and anxiety-related *Gad1* were significantly down-regulated. Meanwhile, there was a decreasing trend in the concentration of GABA in the hippocampal DG region. Next, we supplemented PLP at a dose of 20 mg kg^{-1} day^{-1} to the PY offspring via intraperitoneal injection at three weeks. We found up-regulated expression of *Pdxk* and *Gad1* and restored anxiety-like behaviors. This study suggests that prenatal exposure to PY can disrupt vitamin B6 metabolism, reduce the concentration of PLP, down-regulate the expression levels of *Pdxk* and *Gad1*, inhibit the production of GABA, and ultimately lead to anxiety-like behaviors in offspring.

1. Introduction

Pyridaben (PY), a type of organochlorine pesticide (OCPs), has become the fastest-developing and most widely used acaricide due to its high activity and rapid effects. PY is used to protect farmland, fruit, and vegetable crops. Furthermore, its residues have been detected in various foods [1,2], indicating a broad source of exposure in daily life. Structurally, PY is similar to rotenone, with a cellular inhibitory effect on mitochondrial complexes and NADH dehydrogenase [3]. Despite the adverse impacts observed in animal experiments, such as growth inhibition of uterine epithelial cells [4] and mitochondrial dysfunction of testicular cells [5], the usage of PY is still increasing. In our previous study, it has even been detected in the peripheral blood of pregnant women. Given the chance of exposure in the fetus, the potential health risk of early-life PY exposure could never be neglected.

Anxiety has become one of the most common mental illnesses worldwide [6]. The lifetime prevalence of "any anxiety disorder" in studies with children or adolescents is approximately 15%–20% [7]. The occurrence and development of anxiety are closely related to environmental and genetic factors [8–10]. Additionally, early

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environmental exposure may increase anxiety risk later in life [11]. Mounting epidemiological evidence indicates a positive correlation between exposure to OCPs during pregnancy and neurodevelopmental disorders, including anxiety, impaired motor development, and memory loss [12–15]. Similarly, *in vivo* studies also show increased anxiety-like behavior levels in mice offspring exposed to OCPs during lactation [16]. PY is an organochlorine pesticide (OCPs), similar to rotenone in structure. Although PY has been found to impair mitochondrial function in dopaminergic neurons [17], whether its prenatal exposure affects the neurological behavior of offspring remains unknown.

The metabolic process of the body is quite complex, and its homeostasis is essential to maintain the health of the human body [18]. Moreover, amino acid metabolism is found to be dysregulated under OCPs exposure during pregnancy [19], which is closely associated with various adverse pregnancy outcomes such as abortion [20], birth weight loss [21], and cognitive birth deficits [22] in children. B vitamins boost glutamate's metabolism and ensure the release of neurotransmitters such as GABA and serotonin, which are essential for nervous system signaling. Because 80% of PY is metabolized and binds glucuronic acid and remains in the body, we hypothesized that prenatal PY exposure might affect metabolism and induce neurobehavior toxicity in the offspring.

In this study, we built a prenatal PY exposure model, evaluated the neurobehavior of mice offspring, identified differential metabolites of the brain, and investigated PYs metabolites in mice offspring to explore the effects and underlying mechanisms of prenatal PY exposure on neurobehavior development.

In this study, we demonstrated that prenatal PY exposure inhibited GABA generation by down-regulating the levels of PLP and GAD1 in the brain, resulting in anxiety-like behaviors of mice offspring, which could be restored by supplementation with PLP.

2. Methods and materials

2.1. Animals and treatment

Six-week-old C57BL/6J mice were purchased from the Animal Experimental Center of Nanjing Medical University. The animals were kept under light/dark conditions for 12 h with a temperature of 21 ± 3 °C and a humidity of 52 ± 8%. Food and water are provided *ad libitum*. All mice were adaptively fed for seven days. Then, the female mice were exposed to daily PY gavage. After seven days, mice were mated in cages with a 2:1 ratio of female to male. When the vaginal plug was detected, it was recorded as 0.5 days of gestation (GD 0.5). The mice continued to be gavaged from GD 0.5 to delivery. It has been reported that PY exerts reproductive toxicity [4]. The mice were extremely aggressive during lactation and ate the offspring mice in the study [23]. To obtain adequate offspring for the subsequent experiments, we tried to keep the maternal mouse with 3–4 males and female offspring in the same cage. Afterward, we randomly selected an equal number of mice offspring from each cage in each group for subsequent behavioral tests. Mice offspring on PND1–PND21 are not suitable for behavioral experiments such as OFT and EPM, while five-week-old mice are in a stage that is less affected by the estrous cycle and sex hormones [24] and can stably reflect the neurodevelopmental indicators [25]. Therefore, behavior tests were performed on offspring aged five weeks.

In the National Food Safety Standard–Maximum Residue Limits for Pesticides in Food in China (Chinese National Food Safety Standard, 2019), the ADIs of PY is set to be 0.01 mg kg^{-1} bw^{-1}. In the exposure model, taking the ADI and the NOAEL dose of rat as references, we rounded up the converted dose and finally determined that the low dose is 0.5 mg kg^{-1} day^{-1} and the high dose is 5 mg kg^{-1} day^{-1}. We randomly divided the animals into three groups: the control, the low-dose (0.5 mg kg^{-1} day^{-1}), and the high-dose groups (5 mg kg^{-1} day^{-1}). Next, they were exposed to PY via a daily gavage seven days before pregnancy to delivery (Fig. 1a).

Next, we established a differential metabolite intervention model according to the behavioral and metabolomics results. We chose the dose of 0.5 mg kg^{-1} day^{-1} as the exposure dose for female mice in the intervention model. On day 21 after birth, we randomly divided the offspring of the control group into two groups: one group was injected with saline, named CON, and the other group was injected with pyridoxal 5′-phosphate (PLP), named CON + PLP. Similarly, we randomly divided the offspring of the PY group into two groups: one group was injected with saline, named PY, and the other group was injected with PLP, named PY + PLP. The PLP-supplemented diet at 100 mg kg^{-1} day^{-1} ameliorated neurobehavioral abnormalities in the offspring [26]. Depending on the bioavailability of the mice for the various modes of administration (oral: approximately 20% [27]; intraperitoneal injection: >90% [28]), the intraperitoneal injection dose of PLP in this study was finally determined as 20 mg kg^{-1} day^{-1}. Therefore, the offspring of the CON + PLP and the PY + PLP groups were supplemented with PLP via intraperitoneal injection at a dose of 20 mg kg^{-1} day^{-1} from 21 to 35 days after birth (Fig. 4a).

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University.

2.2. Behavioral tests

Before the behavioral tests, each mouse was stroked for three days. On the day of the experiment, mice were allowed to adapt to the experimental environment for 30 min. Detailed methods of the open field test (OFT), elevated plus maze (EPM), three-chamber test (TCT), morris water maze (MWM), rotarod test, novelty suppressed feeding test (NSF), and light/dark box test (LDT) are provided in the Supplementary materials.

2.3. Metabolomic profiling

We randomly selected six brain tissue samples (36 brains) from each group for metabolic analysis. All samples were analyzed in a randomized fashion. Metabolites were identified according to the comparison of accurate quality and retention time to the metabolite standards. The metabolites detected are listed in Table S1. Detailed methods for sample pre-processing and testing were provided in the Supplementary materials.

2.4. RNA sequencing and real-time qPCR

We randomly selected four brain tissue samples (24 brains) from each group for total RNA extraction. The samples were sent to Novogene Technology Co. Ltd. (Tianjin, China) for library preparation and sequencing. We used real-time qPCR (RT-qPCR) to quantify gene expression. Primer sequences of related genes are shown in Table S2. Detailed methods of RNA sequencing and RT-qPCR are provided in the Supplementary materials.

2.5. Western blot and other experiments

Western blot was conducted to detect the protein levels of GAD1, GAD2, PDXK, and PDXP in mice brains. Histology and immunohistochemistry were used to assess pathological changes. Brain tissue sections were taken from the coronal plane (each section 3–5 μm). Western blot, histology, and immunohistochemistry were performed as described in the Supplementary materials.

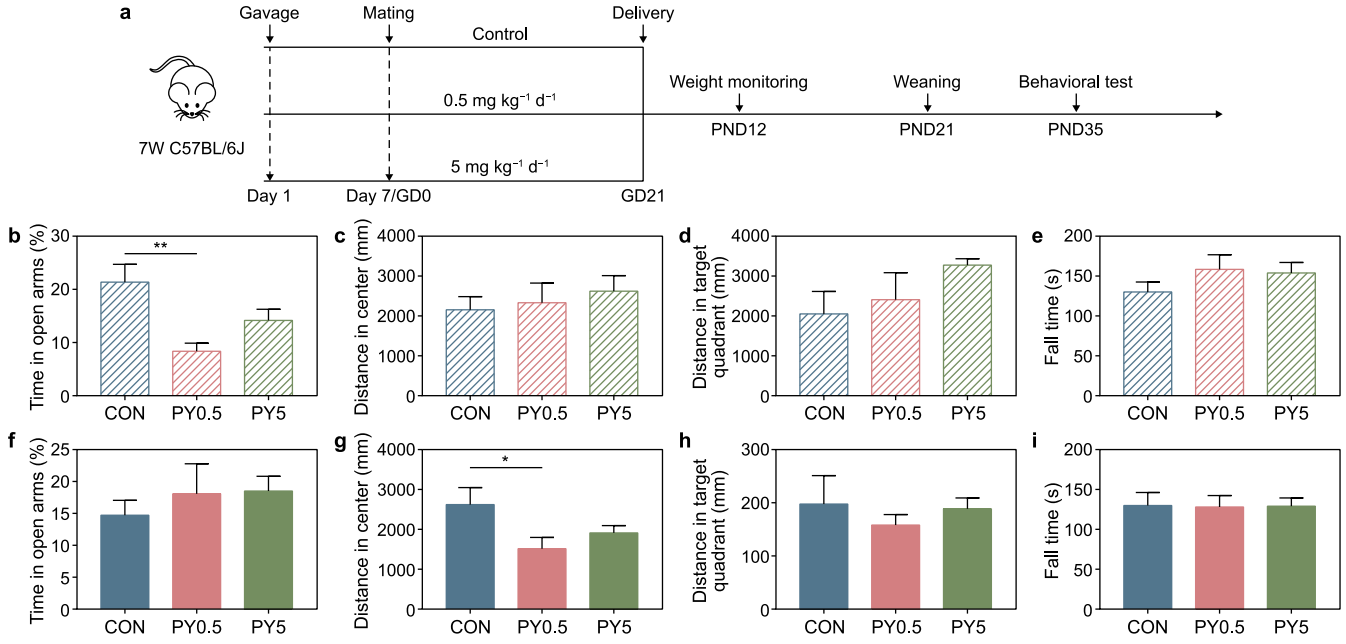

Fig. 1. Effects of exposure to PY during pregnancy on neural behavior of offspring mice. **a**, Experimental design. **b, f**, The elevated plus maze test to examine the anxiety-like behavior by the time in the open arms (%): female (**b**) and male (**f, g**). **c**, The open field test to examine the autonomous exploration behavior by the distance in center (mm): female (**c**) and male (**g**). **d, h**, The Morris water maze (MWM) to test spatial learning by the distance in the target quadrant (mm): female (**d**) and male (**h**). **e, i,** The rotarod test to test the motor coordination of offspring mice by the falling time (s): female (**e**) and male (**i**). Mean ± SEM for $n = 8$ mice per group; $^{*}P < 0.05$; $^{**}P < 0.01$.

2.6. Statistical analysis

All experimental data were analyzed and plotted using GraphPad Prism7. Unpaired Student's *t*-test was used to compare the two data sets, and Welch's correction was added if the homogeneity of variance was not satisfied. Data from more than three groups were compared using a one-way ANOVA and Dunnett multiverb tests or a two-way ANOVA and Sidak multiverb tests. All statistical analyses in this study used $P < 0.05$ as the significance threshold. The experimental results were expressed as mean ± standard error. In the figure, "ns" means no significant difference, and "*" means $P < 0.05$, statistically significant.

3. Results

3.1. Effects of prenatal PY exposure on body and organ weight of mice offspring

The offspring of each group were weighed every day from day 12 to day 35 after birth. As shown in the Supplementary figures (Fig. S1), no significant difference was observed in body weight between the control and treatment groups. After the behavioral tests, the mice were euthanized by neck amputation, and the heart, liver, kidney, brain, and testis/uterus were collected and weighed. Compared to the control group, the uterus coefficient of the female offspring was significantly increased ($P = 0.030$). However, no significant changes were found in the coefficients of other organs.

3.2. Effects of prenatal PY exposure on the neurobehavior of mice offspring

To explore whether prenatal PY exposure affects neurobehavior development, we conducted tests including EPM, OFT, MWM, rotarod test, and TCT to evaluate neurobehavior in 5-week-old offspring.

During the EPM test, the time spent in the open arms of the female offspring in the low-dose group was significantly shorter than the control group ($P = 0.002$) (Fig. 1b). However, no significant change was found between the control and treatment groups of the male offspring (Fig. 1f). The results suggested that PY may increase the anxiety-like behavior levels of female offspring mice.

During the OFT test, we observed no significant difference in the center moving distance of female offspring mice in the low-dose group compared with the control group (Fig. 1c). However, the center moving distance of male offspring mice in the low-dose group was significantly shorter than that in the control group ($P = 0.048$) (Fig. 1g), indicating that PY disrupted exploration competence, which is an indirect proof of anxiety-like behaviors, of male offspring.

The MWM results showed that the time spent in the target quadrant and the time to cross the target region were not significantly abnormal in the offspring mice of the treatment groups (Fig. 1d and h). The results of the rotarod test showed that the fall time was roughly the same in all groups (Fig. 1e and i). We also conducted the TCT test to assess sociability in offspring mice, and a significant discrepancy was not observed in the sociability and social novelty stages between the two groups (Fig. S2).

According to the above results, we preliminarily concluded that prenatal exposure to 0.5 mg kg^{-1} day^{-1} of PY induced anxiety-like behaviors in offspring mice.

3.3. Effects of prenatal PY exposure on brain metabolism of offspring mice

To investigate the effects of prenatal PY exposure on metabolism, we examined the brain metabolome within the offspring mice. Since we observed significant anxiety-like behaviors in the female offspring of the low-dose group, we conducted subsequent experiments with the offspring of this group. A total of 131 metabolites were detected, including amino acids, fatty acids,

carbohydrates, and other small-molecular substances (Table S1). There were 22 significantly differential metabolites observed in the low-dose group ($P < 0.1$), of which ten metabolites increased while the other 12 decreased (Fig. 2a). We performed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on 22 different metabolites, and found that the vitamin B6 metabolism pathway was significantly down-regulated ($P = 0.006$) (Fig. 2b). Compared with the control group, PLP the active form of vitamin B6, was significantly decreased ($P = 0.001$), while the pyridoxal (PL) that produces PLP was significantly increased ($P = 0.001$) (Fig. 2a). Next, we used ELISA to measure the concentration of vitamin B6 in the brain tissue of female offspring mice. The results showed that vitamin B6 concentrations were significantly increased after prenatal PY exposure ($P = 0.0418$) (Fig. S3A), which was consistent with the metabolomics results. This suggested that exposure to PY during pregnancy leads to abnormal vitamin B6 metabolism in the brain of their offspring.

3.4. Effects of prenatal PY exposure on brain gene expression of offspring mice

To explore the effects of prenatal PY exposure on gene expression, we conducted RNA-seq on the brain of five-week-old offspring mice. A total of 28,649 genes were detected and were included in subsequent studies. There were 515 up-regulated and 553 down-regulated genes (Fig. 2c). Gene oncology (GO) enrichment analysis was performed on all the differential genes. The results showed that down-regulated genes primarily involved with neurodevelopment, such as regulation of synaptic transmission and GABAergic synapse. The up-regulated genes are mainly involved in inflammatory activation, such as the regulation of cytokine production involved in inflammatory and cellular responses to interleukin-1 (Fig. 2d). Next, we used RT-qPCR to detect anxiety-related genes' expression levels in these pathways, including *Shank3*, *Glud1*, *Gad1*, *Il-6*, *Il-8*, and *Il-17a*. It was found that the expression levels of *Gad1* and *Shank3* genes were significantly down-regulated in the low-dose exposure group ($P = 0.041$ and 0.002, respectively) (Fig. S3b), while the inflammatory pathway-related genes such as *Il-8* and *Il-17a* were significantly up-regulated ($P < 0.001$ and 0.019, respectively) (Fig. S3c). We also detected the expression of genes in the vitamin B6 metabolism pathway, including *Pdxk*, *Pdxp,* and *Pnpo*. The results revealed that the expression of *Pdxk* and *Pdxp* were significantly down-regulated ($P = 0.022$ and $P < 0.001$, respectively) (Fig. S3d). The expression levels of the genes mentioned above were significantly altered in the high-dose group.

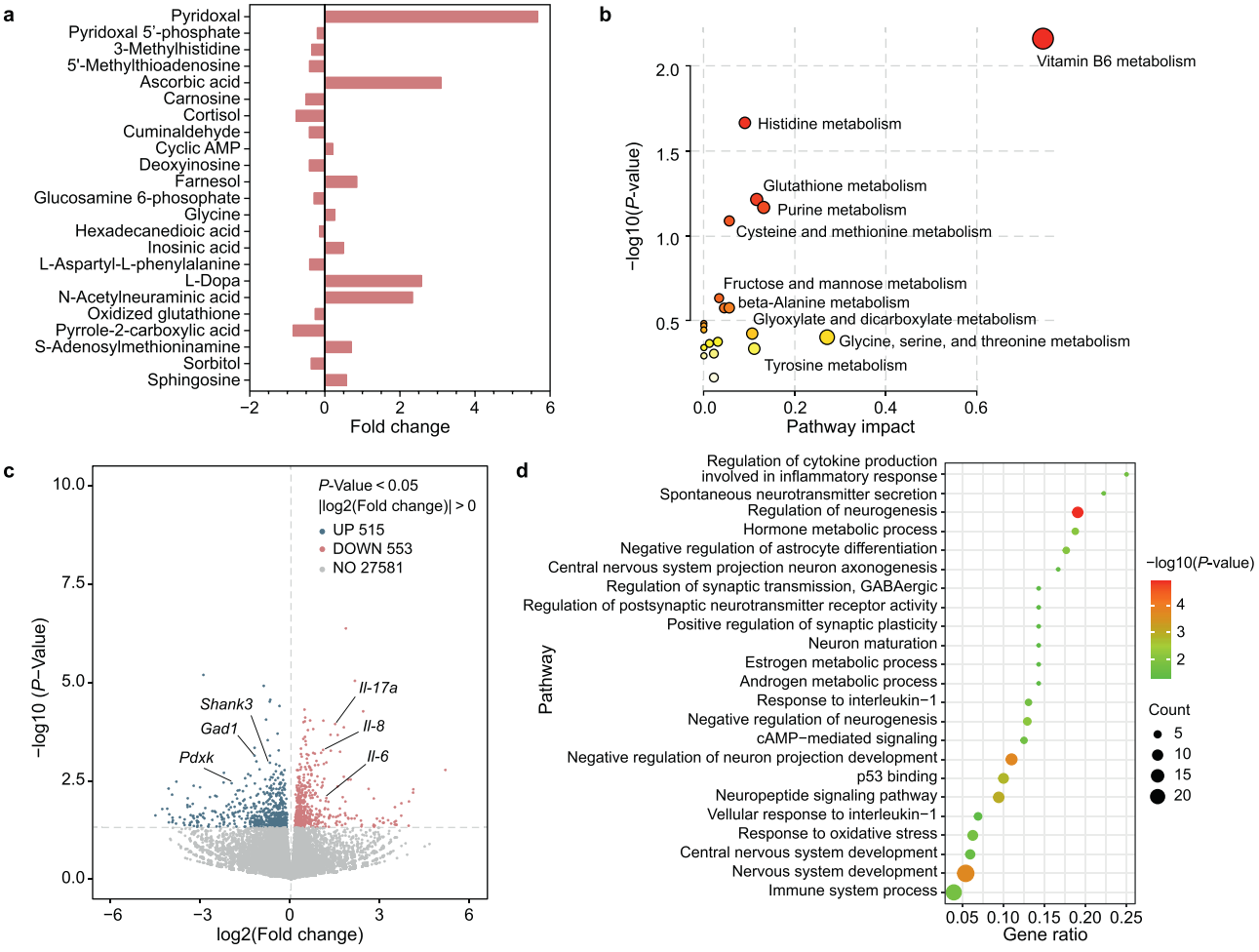

Fig. 2. Effects of exposure to PY during pregnancy on brain metabolism and transcriptomics in offspring mice. **a**, The fold change of differential metabolites in brain tissue of female offspring mice treated with PY (PY−F and Control-F). **b**, Metabolic functions of these differential metabolites according to the Kyoto Encyclopedia of Genes and Genomes (KEGG). **c**, Volcano plots showed differentially expressed genes. **d**, GO analysis of differentially expressed genes induced by PY (PY−F and Control-F).

3.5. Integrated analysis of metabolites and genes in the brain of offspring mice

To integrate the multi-omics data, we established a link between the metabolites and the genes via the KEGG pathway enrichment analysis, demonstrating the important role of the vitamin B6 metabolic pathways (Fig. 3). PL, the center of vitamin B6 metabolism, is transformed into PLP under the action of *Pdxk*. Due to the down-regulation of *Pdxk*, the transformation was blocked, resulting in the reduction of PLP and the down-regulation of *Pdxp,* which hydrolyses PLP back to PL. This leads to the accumulation of PL. To solve this accumulation and maintain the homeostasis of metabolism, PL is metabolized to 4-pyridoxate under the actions of *Aox4* and *Aox2*, which was mostly excreted from the body through the feces and partly synthesized to succinic acid. Given the above results, we speculated that PY could inhibit the expression of *Pdxk*, block the transformation of PL, and reduce the production of PLP, leading to anxiety-like behaviors in offspring mice.

3.6. Effects of PLP supplementation on body and organ weight of offspring mice

According to the behavioral and multi-omics analysis results, we selected PLP to construct an intervention animal model (Fig. 4a). We monitored the body weight of the offspring from day 12 to day 35 after birth. No significant changes were found in the body weight of the offspring mice in all groups (Figs. S4a and S4b). After the behavioral experiment, the mice were dissected, and their heart, liver, kidney, brain, and testis/uterus samples were extracted to measure organ weight. The results showed that the organ coefficient did not change significantly (Figs. S4c–f).

3.7. Effects of PLP supplementation on anxiety-like behaviors of offspring mice

In the exposure model, we found that prenatal PY exposure led to anxiety-like behaviors; therefore, we focused on assessing anxious behavior and performed the NSF, LDT, and EPM tests to verify whether PLP intervention can reverse the anxiety-like behaviors in offspring mice. During the NSF test, compared to the control group, the latency of feeding of the female offspring mice in the PY group was significantly increased and recovered to the control level after PLP supplementation ($P < 0.001$) (Fig. 4b). The latency of feeding of the male offspring mice was longer than that of the PY group ($P = 0.111$); however, there was no statistical difference compared to the control group, and they returned to the control level after supplementing with PLP ($P = 0.001$) (Fig. 4f). Meanwhile, the food consumption among groups had no significant difference (Fig. 4c and g).

During the LDT test, the latency to enter the dark box of all offspring in the PY group was increased compared to the control group ($P = 0.442$ and 0.059, respectively) and decreased after supplementing with PLP ($P = 0.276$ and 0.109, respectively); however, there was no statistical difference compared to the control group (Fig. 4d and h). Furthermore, the number of transitions of both the female and the male offspring in the PY group was decreased compared with the control group ($P = 0.056$ and 0.325, respectively). However, there was no statistical difference compared to the control group and no significant difference after PLP supplementation (Fig. 4e and i). During the EPM test, the control and treatment groups observed no significant difference in retention time, movement distance, and the number of offspring entering the open arms (Fig. S5). The above results verified that prenatal PY exposure induced anxiety-like behaviors in offspring mice, and PLP was found to restore the effects of PY.

3.8. Effects of PLP supplementation on the brain pathology and immunohistochemistry of offspring mice

Moreover, PLP supplementation alone does not affect the behavior of mice; therefore, we focused on the other three groups. We performed Nissl staining to explore whether exposure to PY during pregnancy and PLP supplementation affects brain tissue pathology in offspring mice. The results indicated that the number of Nissl bodies in the DG region of the female and male offspring mice in the PY group decreased significantly ($P = 0.014$ and 0.011, respectively); however, there was no sign of recovery in the PY + PLP group (Figs. S6a–d). Meanwhile, we did not find any

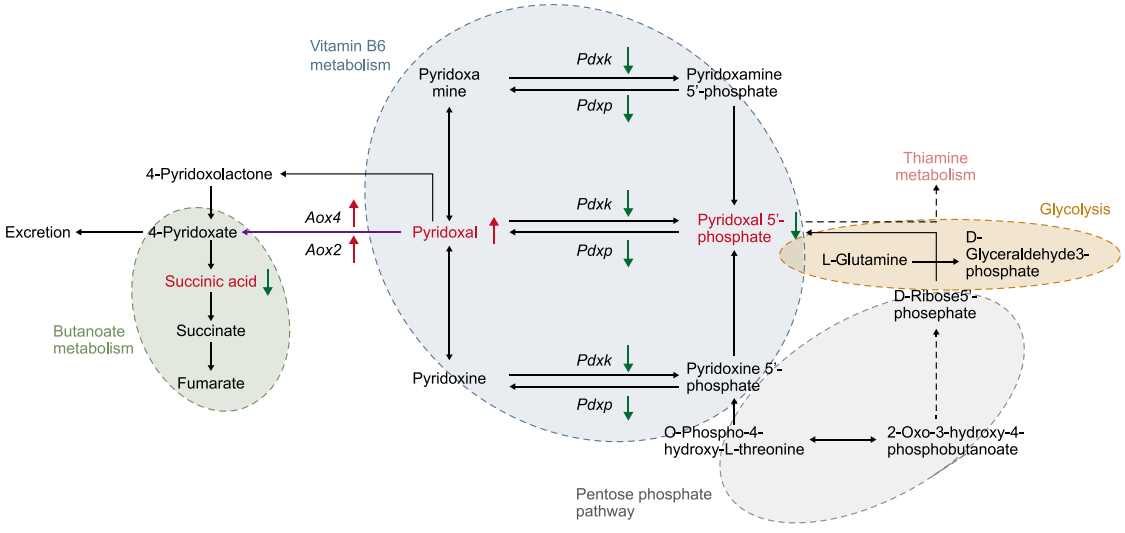

Fig. 3. Associations between metabolites and genes in the brain tissue of offspring mice. Metabolic pathways were constructed from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. The black text represents metabolites. The oval region represents metabolic pathways, including vitamin B6 metabolism (blue), butanoate metabolism (green), glycolysis metabolism (yellow), pentose phosphate pathway (gray), and thiamine metabolism (pink). Black italic text indicates genes. The red text represents the metabolites detected in this work. The red and green down arrows indicate metabolites and genes up or down, respectively, compared to the control group.

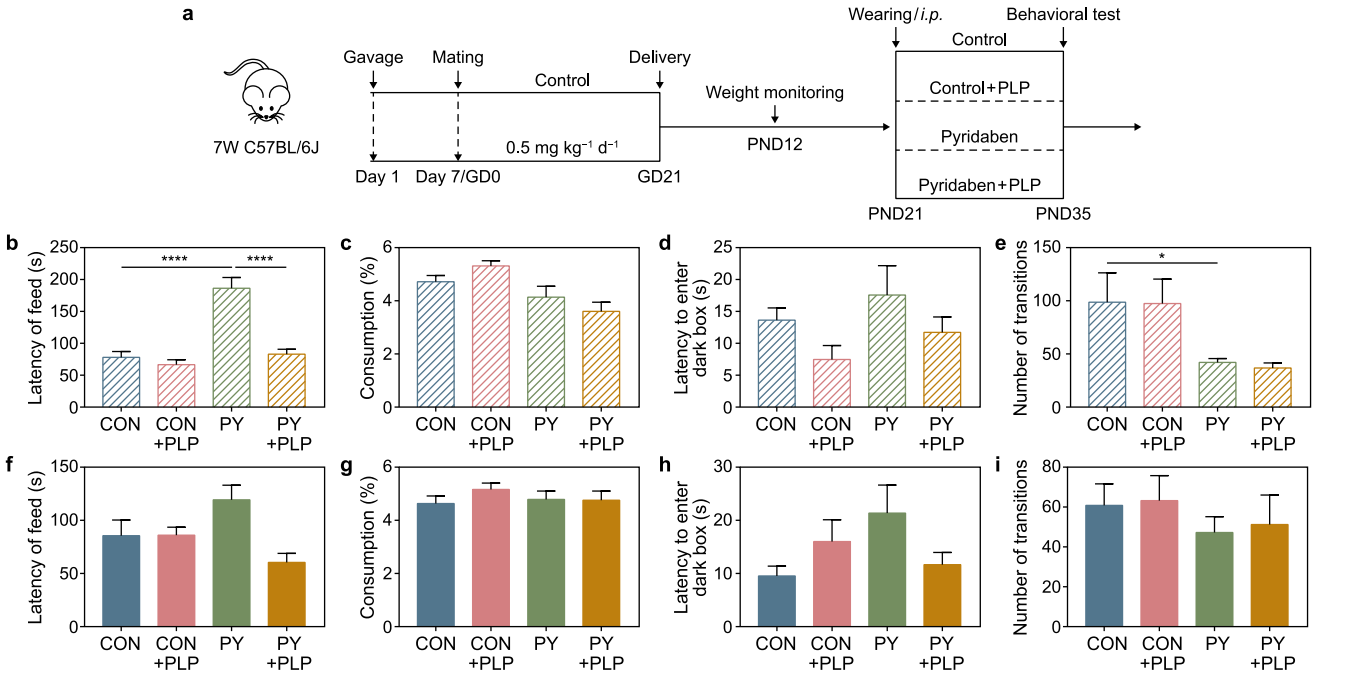

Fig. 4. Effects of PLP supplementation on anxiety-like behavior of offspring induced by exposure to PY during pregnancy. **a**, Experimental design. **b**–**c**, **f**–**g**, the novelty suppressed feeding test to test the anxiety-like behavior by the latency of feed (s) and consumption (%): female (**b**–**c**) and male (**f**–**g**). **d**–**e**, **h**–**i**, The light and dark box to test the anxiety-like behavior by the number of latency(s) and the number of transitions: female (**d**–**e**) and male (**h**–**i**). Mean ± SEM for $n = 8$ mice per group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

statistical differences in the number of Nissl bodies in the amygdala, cortex, and hippocampal CA1 and CA3 regions of the offspring among the treatment and control groups (Fig. S7).

To explore whether the levels of GABA changed, we conducted immunohistochemical experiments (Figs. S6e and S6f). The results showed that in the hippocampus DG region, the number of GABA-positive cells in the female PY group was lower than in the control group ($P = 0.431$), while the PY + PLP group was higher than the PY group ($P = 0.612$); however, there was no statistical difference compared to the control group (Fig. S6g). Similarly, no significant difference was observed in the male offspring mice (Fig. S6h).

3.9. *Effects of PLP supplementation on gene and protein levels in the brain of offspring mice*

To further examine the molecular mechanism of the anxiety-like behaviors induced by prenatal PY exposure, we investigated mRNA expression levels in the brains of offspring mice after PLP supplementation. Compared with the control group, the expression of *Gad1* and *Pdxk* in female offspring of the PY group was significantly down-regulated ($P = 0.047$ and 0.049, respectively) and returned to normal after PLP supplementation ($P < 0.001$ and 0.023, respectively) (Fig. 5a). However, the gene expression levels of the male offspring mice of each group showed no significant difference (Fig. 5b). Next, we investigated the protein levels in the brain. We found that the levels of GAD1 and PDXK were significantly decreased in the female offspring mice of the PY group ($P = 0.012$ and 0.032) and restored in the PY + PLP group ($P = 0.002$ and 0.045) (Fig. 5c and e), while no significant change was observed among male offspring mice (Fig. 5d and f).

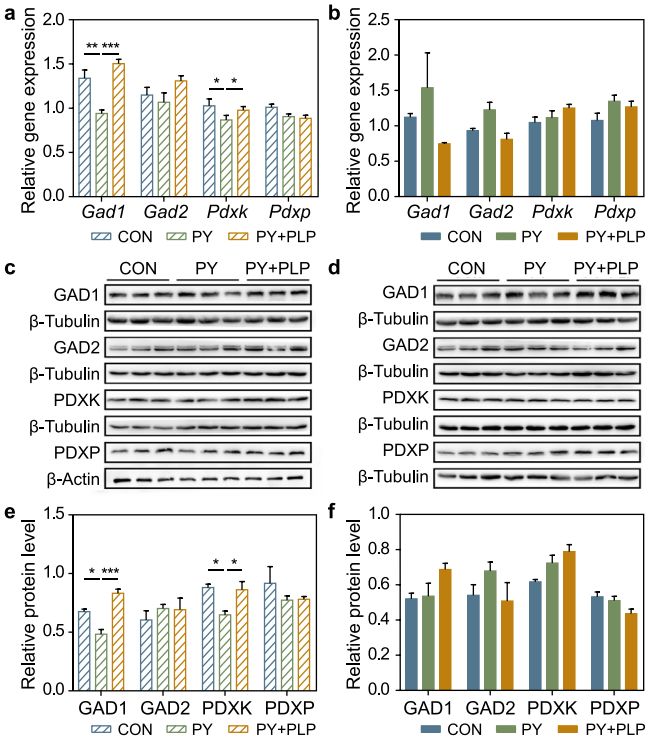

Fig. 5. Analysis of gene and protein expression in the brain of offspring mice. **a**–**b**, Gene expression in the brain of offspring mice: female (**a**) and male (**b**). **c**–**d**, Representative Western blot picture of proteins PDXK, PDXP, GAD1, and GAD2 in the brain of offspring mice with the control β-Tubulin or β-Actin: female (**c**) and male (**d**). **e**, The PDXK and GAD1 were significantly changed in female offspring mice. **f**, The PDXK, PDXP, GAD1, and GAD2 was no significantly change in male offspring mice. The data shown are the mean ± SEM. The data were analyzed by unpaired two-tailed Student's *t*-test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

In this study, we assessed the neurobehavior of offspring mice,

described the metabolome and transcriptome characteristics, analyzed the expression of genes and proteins in related biological pathways, and verified the role of PLP supplementation in reducing the adverse effects of prenatal PY exposure using exposure and intervention experiments. Our results showed that exposure to PY during pregnancy increased anxiety-like behavior levels and decreased brain PLP concentrations in offspring mice. In contrast, anxiety-like behaviors were restored after supplementation with PLP. To our knowledge, this is the first study to evaluate the effects of prenatal PY exposure on the neurobehavioral development of offspring mice.

Nearly 20% of the world's population suffers from anxiety [29,30], and women are twice as likely to suffer from anxiety than men [31]. To date, many studies have indicated that the occurrence of anxiety-like behaviors in childhood and/or adolescence can be traced back to environmental exposure in utero [32–34]. For example, prenatal exposure to OCPs can lead to increased abnormal reflexes, reduced motor performance, and cognitive deficits in children [35–37]. Similarly, numerous animal studies have shown that prenatal exposure to these pesticides leads to increased anxiety-like behaviors levels in offspring mice [16,38,39]. Consistent with this, our results also suggested that prenatal PY exposure in mice leads to anxiety-like behaviors in offspring.

It was found that exposure to PY during pregnancy down-regulated the levels of GAD1. Although there was no statistical difference, GABA levels tended to decrease compared with controls. GABA is one of the earliest and most important neurotransmitters in the brain, providing the initial excitatory drive for neural activity and establishing a balance between inhibitory and excitatory synapses. The breakdown of the GABAergic inhibitory circuit and the resulting excitatory/inhibitory imbalance has been widely reported as one of the mechanisms of anxiety [40,41]. Further studies have also proven that increasing or decreasing the synthesis of GABA to maintain the excitatory/inhibitory balance reduces or even reverses anxiety-like behavior [42]. Furthermore, the ERK1/2-GAD1-GABA cascade was recently reported to be involved in this process [43]. Therefore, it is important to clarify the specific role of GABA metabolism during prenatal PY exposure-induced anxiety-like behaviors.

In our study, exposure to PY during pregnancy inhibits the transformation of PL to PLP, resulting in reduced PLP. The content of GABA primarily depends on the activity of PLP and GAD1. The deficiency of PLP causes a decrease in GAD1 activity and inhibits glutamate's decarboxylation, leading to a decrease in GABA production [44]. This eventually leads to GABA neurotransmission disorder, causing anxiety, epilepsy, and other neurological diseases [45]. In addition, PLP inhibits GABA binding to synaptic membrane receptors [46]. Moreover, GABA and some GABA receptor agonists have protective effects on PLP-induced seizures [47], which can be speculated to affect GABA receptors. PDXK and PDXP mainly regulate the transformation of PL to PLP, the former promotes PL phosphorylation to PLP, and the latter dephosphorylates PLP to PL. The loss of PDXP has been shown to alter the balance between excitatory and inhibitory transmission at specific synapses in the body. For example, in the PDXP-KO mouse model, PLP levels increased threefold, GABA levels increased by 20%, and the mice showed mild anxiety-like behaviors [48], suggesting that increasing PLP levels increase GABA production. In addition, the level of L-serine and glycine were decreased in PDXP-KO mice [48]. Our results showed that PDXK expression was down-regulated, and the concentrations of L-serine and glycine were increased in offspring mice after exposure to PY during pregnancy. Serine racemase and hydroxymethyl transferase are PLP-dependent enzymes that produce D-Serine and glycine. Both are associated with excitatory activity in the brain. All of these changes contributed to the anxiety-like behaviors observed in offspring mice. Considering that PDXK and PDXP may not have completely opposite effects, it is necessary to further study the metabolic characteristics of the brain and neurobehavioral phenotypes of offspring mice after overexpression of PDXK.

In addition, we observed anxiety-like behaviors in female offspring mice but not in male offspring. Studies have reported that changes in circulating estrogen levels during the reproductive cycle are associated with the incidence of anxiety in women [49,50]. Although estrogens and their sulfate and phosphate forms have been shown to be active on various PLP-dependent enzymes [51,52], there is no research on sex differences between PLP and anxiety-like behaviors. Therefore, further studies are needed to clarify the role of PLP in the sexual dimorphism of anxiety-like behaviors.

Our study, combined with the above results, showed that prenatal PY exposure induced anxiety-like behaviors in offspring mice. We only assessed the effect of PLP supplementation on anxiety-like behaviors in offspring mice. More studies are needed in the future to explore the role of *Pdxk* in brain metabolism and neurobehavioral abnormalities caused by exposure to PY during pregnancy in offspring mice.

5. Conclusion

In the present study, we demonstrated that prenatal PY exposure inhibited GABA generation by down-regulating the levels of PLP and GAD1 in the brain resulting in anxiety-like behaviors of offspring mice which was restored by supplementation with PLP. Further studies are still needed to explore the role of *Pdxk* in prenatal PY exposure-induced metabolic disorders of the brain, as well as the neurobehavioral abnormalities of offspring mice.

CRediT authorship contribution statement

Xingwang Ding: Writing- Original draft, Reviewing & Editing; **Ya Wen:** Investigation, Validation; **Xuan Ma:** Software, Formal analysis, Data curation; **Yuepei Zhang:** Investigation; **Yuting Cheng:** Investigation; **Zhaofeng Liu:** Validation; **Weiyue Hu:** Conceptualization, Methodology, Supervision, Funding acquisition; **Yankai Xia:** conceptualization, funding acquisition, resources, supervision, data curation, capital source. **Xingwang Ding, Ya Wen and Xuan Ma** contributed equally to this work. The authors declare they have no actual or potential competing financial interests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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